Amendments to the Specification:

Please replace paragraph [0050] with the following amended paragraph.

[0050] FIGURE 1 shows a UDG-cloning cassette ("cloning linker") and a scheme of vector preparation for ligation-independent cloning (LIC) using the nicking endonuclease N. BbvC IA. FIGURE 1A. UDG-cloning cassette. *Sac I* and nicking enzyme sites used in vector preparation are labeled. FIGURE 1B. Scheme of vector preparation for LIC using nicking endonuclease N. BbvC IA. SEQ ID NOS:21-25, and 31-33 are shown.

Please replace paragraph [0051] with the following amended paragraph.

[0051] FIGURE 2 illustrates the Method S joining method using Bbs I and Bsa I as the Type IIS restriction enzymes. SEQ ID NOS:26-29, and 34-36 are shown.

Please replace paragraph [0058] with the following amended paragraph.

[0058] FIGURE 9 is a flowchart showing a GeMS algorithm. Figure 9A shows initial steps in the algorithm. Figure 9B shows subsequent steps in the algorithm.

Please replace paragraph [0068] with the following amended paragraph.

[0068] ~~FIGURE 18 shows~~ FIGURES 18A and 18B show restriction sites and synthons used in construction of a synthetic Epothilone PKS gene.

Please replace paragraph [0089] with the following amended paragraph.

[0089] The term "junction edge" is used to describe the region of a synthon that is joined to an adjacent synthon (e.g., by formation of compatible ligatable ends in each synthon). Thus, reference to "a ligatable end at a junction end" of a synthon means the end that is (or will become) ligated to the compatible ligatable end of the adjacent synthon. It will be appreciated

that in a construct with five or more synthons, most synthons will have two junction edges. The junction edge(s) being referred to will be apparent from context. A sequence motif or restriction enzyme site is "near" the nucleotide sequence encoding an amino-or carboxy-terminus of a PKS domain in a module when the motif or site is closer to the specified terminus (boundary) than to the terminus (boundary) of any other domain in the module. A sequence motif or restriction enzyme site is "near" the nucleotide sequence encoding an amino-or carboxy-terminus of a PKS module when the motif or site is closer to the specified terminus (boundary) than to the terminus of any domain in the module. The boundaries of PKS domains can be determined by methods known in the art by aligning the sequence of a subject domain with the sequences of other PKS domains of a similar type (e.g., KS, ER, etc.) and identifying boundaries between regions of relatively high and relatively low sequence identity. See Donadio and Katz, 1992, "Organization of the enzymatic domains in the multifunctional polyketide synthase involved in erythromycin formation in Saccharopolyspora erythraea" *Gene* 111:51-60. Programs such as BLAST, CLUSTALW and those available at ~~http://www.nii.res.in/pksdb.html~~ www.nii.res.in/pksdb.html can be used for alignment. In some embodiments, a motif or restriction enzyme site that is near a boundary is not more than about 20 amino acid residues from the boundary.

Please replace paragraph [0092] with the following amended paragraph.

[0092] As used herein, "restriction endonuclease" or "restriction enzyme" has its usual meaning in the art. Restriction endonucleases can be referred to by describing their properties and/or using a standard nomenclature (see Roberts et al., 2002, "A nomenclature for restriction enzymes, DNA methyltransferases, homing endonucleases and their genes," *Nucleic Acids Res.* 31:1805-12). Generally, "Type II" restriction endonucleases recognize specific DNA sequences and cleave at constant positions at or close to that sequence to produce 5'-phosphates and 3'-hydroxyls. "Type II" restriction endonucleases that recognize palindromic sequences are sometimes referred to herein as "conventional restriction endonucleases." "Type IIA" restriction endonucleases are a subset of type II in which the recognition site is asymmetric. Generally, "Type IIS" restriction endonucleases is a subset of type IIA in which at least one cleavage site is outside the recognition site. As used herein, reference to "Type IIS" restriction enzymes, unless

otherwise noted, refers to those Type IIS enzymes for which both DNA strands are cut outside the recognition site and on the same side of the restriction site. In one embodiment of the invention, Type IIS enzymes are selected that produce an overhang of 2 to 4 bases. Exemplary restriction endonucleases include Aat II, Acl I, Afe I, Afl II, Age I, Ahd I, Alw 26I, Alw NI, Apa I, Apa LI, Asc I, Ase I, Avr II, Bam HI, Bbs I, Bbv CI, Bci VI, Bcl I, Bfu AI, Bgl I, Bgl II, Blp I, Bpl I, Bpm I, Bpu 10I, Bsa I, Bsa BI, Bsa MI, Bse RI, Bsg I, Bsi WI, Bsm BI, Bsm I, Bsp EI, Bsp HI, Bsr BI, Bsr DI, Bsr GI, Bss HII, Bss SI, Bst API, Bst BI, Bst EII, Bst XI, Bsu 36I, Cla I, Dra I, Dra III, Drd I, Eag I, Ear I, Eco NI, Eco RI, Eco RV, Fse I, Fsp I, Hin dIII, Hpa I, Kas I, Kpn I, Mfe I, Mlu I, Msc I, Nco I, Nde I, Ngo MIV, Nhe I, Not I, Nru I, Nsi I, Pac I, Pci I, Pfl MI, Pme I, Pml I, Psh AI, Psi I, Pst I, Pvu I, Pvu II, Rsr II, Sac I, Sac II, Sal I, San DI, Sap I, Sbf I, Sca I, Sex AI, Sfi I, Sgf I, Sgr AI, Sma I, Smi I, Sml I, Sna BI, Spe I, Sph I, Srf I, Ssp I, Stu I, Sty I, Swa I, Tat I, Tsp 509I, Tth 111I, Xba I, Xcm I, Xho I, Xmn I, those listed in Table 2, and ~~others.e.g., http://rebase.neb.com~~ others, e.g., //rebase.neb.com).

Please replace paragraph [0115] with the following amended paragraph.

[0115] In considering codon preferences, preference tables may be obtained from publicly available sources or may be generated by the practitioner. Codon preference tables can be generated based on all reported or predicted sequences for an organism, or, alternatively, for a subset of sequences (e.g., housekeeping genes). Codon preference tables for a wide variety of species are publicly available. Tables for many organisms are available at through links from a site maintained at the Kazusa DNA Research Institute ~~(http://www.kazusa.or.jp/codon/)~~ (www.kazusa.or.jp/codon/). An exemplary codon preference for *E. coli* is shown in Table 1. Codon tables for *Saccharomyces cerevisiae* can be found in http://www.yeastgenome.org/codon_usage.shtml. In the event that no codon table is available for a particular host, the table(s) available for the most closely related organism(s) can be used.

Please replace paragraph [0310] with the following amended paragraph.

[0310] The Racoon system is implemented using the following software components: Phred, Phrap, Cross_Match (Ewing B, Hillier L, Wendl M, Green P: Base calling of automated

sequencer traces using phred. I. Accuracy assessment. Genome Research 8, 175-185 (1998); Ewing B, Green P: Basecalling of automated sequencer traces using phred. II. Error probabilities. Genome Research 8, 186-194 (1998); Gordon, D., C. Desmarais, and P. Green. 2001. Automated Finishing with Autofinish. Genome Research. 11(4):614-625); Python 2.2 as integration and scripting language (Python Essential Reference, Second Edition by David M. Beazley); GeMS Application Programming Interface (Kosan proprietary software); Apache Web Server version 2.0.44 (httpd.apache.org) ~~(http://httpd.apache.org)~~; and Red Hat Linux Operating System version 8.0 (www.redhat.com) ~~(http://www.redhat.com)~~.

Please replace paragraph [0339] with the following amended paragraph.

[0339] The results of the gene design for the four common variants ([KS+AT+ACP]; [KS+AT+ACP + KS]; [KS+AT+ACP + KS +DH]; [KS+AT+ACP + KS+DH+ER] of PKS modules are shown in Figure 4 and Tables 7-11. The positions of the restriction sites are referenced to the homologous amino acid target sites within a domain where possible, and to module 4 of the 6-DEBS gene or protein (which contains all six of the common domains). For the latter, numbering of the amino acid and nucleotide sequence used for reference begins at the first residue of the EPIAIV found on the N-terminal edge of the KS domain; homologous motifs are found at the N-terminal edges of all 140 KS domains in the sample.

Please replace Table 7 with the following amended table.

TABLE 7

RESTRICTION SITES NEAR DOMAIN EDGES

Restriction Enzyme	Domain / Terminal Orientation	Nucleotide Position of site in ery mod4 *	AA Sequence near site in ery mod4	SEQ ID NO:	Amino acid motif in ery mod4
Spe I	ACP (C)	54 bp before KS			VG-not conserved
Mfe I	KS (N)	5-10	PIAIVG	12	PIA
Kpn I	KS (C)	1243-1248	GTNAHV	13	GT
Msc I	AT (N)	1590-1595	PGQGAQ	14	GQ
Pst I	AT (C)	2611-2616	PRPHRP	15	PR-not conserved
BsrB I	ER (N)	4075-4080	PLRAGE	16	PL
Age I	KR (N)	5029-5034	TGGTGT	17	TG (initial TG)
Xba I	ACP (C)	6001-6006	FADSAP	18	FA (not conserved) from DEBS2 near terminus

* Numbering for each module begins at the N-terminus of the KS domain taken to be the amino acid at the site homologous to that of the glutamate (E) of the E-P-I-A-I-V (SEQ ID NO:19) of module 4 of erythromycin.

Please replace paragraph [0341] with the following amended paragraph.

[341] An Msc I site is incorporated near the left edge of the AT coding sequence (nt 1590-1595) at the site of the GQ dipeptide found in 100% of the sampled ATs. A Pst I site was placed at the right side of the AT (nt 2611-2617) at a position where Pst I and Xho I had been previously placed without loss of functionality after domain swaps. This variable sequence region is identified in many modules by a Y-x-F-x-x-x-R-x-W (SEQ ID NO:20) motif where "x" is any amino acid; in others, alignments always produce a well-defined equivalent position. The two amino acids to the immediate right (C-terminal to W) of this motif are modified to introduce the Pst I site.

Please replace paragraph [00364] with the following amended paragraph.

[0364] As a model system to develop this technology, we used a protein that was labelled with two different epitope tags ~~(cmyc-AtoC-FLAG-BRS-His)~~ (cmyc-AtoC-FLAG™-BRS-His) on either end (the 55 kDa AtoC). This provided a protein in which the two tags are present in a known ratio.

Please replace paragraph [00365] with the following amended paragraph.

[0365] In our initial experiments, we had difficulties obtaining reproducible ratios of two Mab's bound to the protein after Western blot, especially with sub-microgram quantities. We therefore made the effort to develop the methods of analysis needed using dot-blots of ~~cmyc-AtoC-FLAG~~ cmyc-AtoC-FLAG™. In the data shown below, two fluorescently labelled antibodies (cymc-AlexaFluor488 and ~~FLAG-Cy5~~ FLAG™-Cy5) were used simultaneously to quantitate a dot-blot of the AtoC construct mentioned above. The blot was scanned using a Typhoon 9410 Fluorescent Imager, and analysis was performed using ImageQuant software. Results are shown in Table 15.